SUBMISSION OF CERTIFIED TRANSLATION

In a previous Office Action dated October 14, 2009, claims 1, 4, and 6-12 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Sturla et al. (British Journal of Cancer (2003) 89, 1276-1284). The Examiner argued that Sturla shows that FGFRIII3S protein, a variant of FGFR3, is expressed at very high levels in bladder cell cancer line RT112. This rejection was subsequently withdrawn over arguments that the FGFRIII3S protein is not the same as FGFR3b or FGFR3c, the usual forms of FGFR3. Although the Applicant believed this to be true at the time the argument was made, it has since been discovered that the technique of the current application may identify FGFRIII3S as well as FGFR3b or FGFR3c. Accordingly, we are forced to withdraw our argument for patentability of claims 1, 4, and 6-12 over Sturla.

Therefore, we hereby submit a certified English translation of P200300708, the Spanish priority document. Sturla was published on 30 September 2003, while the earliest priority date of the current application is March 26, 2003. Therefore, since the claim to foreign priority has now been perfected by submission of the translated priority document, Sturla is not available as prior art against the current application. We attach a citation of Sturla giving the correct publication date. See Exhibit A.

REJECTIONS OVER PRIOR ART

In the currently pending Office Action, claims 1,4, and 6-12 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Cappellan (Nature Genetics Vol. 23, cited in the IDS) in view of KSR and Sturla. We note that Sturla has been effectively removed as a reference, for reasons discussed above. The Examiner argues that Cappellen discloses that there is a significant difference in mRNA levels of FGFR3b in patients bladder carcinomas from those without bladder carcinomas, and argues that this makes FGFR3b an excellent marker for bladder carcinomas. The Examiner relies on Sturla to teach that Western blotting and immunoprecipitation are well known in the field. Sturla also teaches that FGFR3b and FGFR3c are the normal forms of the FGFR3 protein.

Claim 1 relates to detection or quantification of the FGFR3 protein.

Capellen discloses that activating mutations in the FGFR3 gene are detected in bladder carcinomas, and also discloses specific point mutations in the *FGFR3* gene which are detected in bladder carcinomas. In other words, the teaching of Capellen is directed to the use of some **specific point mutations in the *FGFR3* gene** as a marker of bladder carcinoma in an individual. The method disclosed in the current application differs from the teaching of Capellen in that what is used as a diagnostic marker of bladder transitional cell carcinoma in an individual is the **expression of the FGFR3 protein** in a sample from the individual.

Diagnostic methods based on the detection and identification of gene mutations, are not very sensitive or specific due to the detection of false positives and negatives, and, consequently, said methods are not very reliable.

Sensitivity and specificity of diagnostic methods based on the detection and identification of gene mutations associated with polygene diseases (e.g., cancers) is not reliable because a mutation may not have consequences in the expression and functionality of the protein, thus being a polymorphism with no phenotype consequences. Additionally, in a tumor, not all the cells necessarily contain the mutation; thus, a mutation in a DNA or RNA sequence may not be detected. Finally, diseases such as cancer may be controlled by multiple genes. Even if a mutation connected to cancer does exist, other polymorphisms or mutations in other genes may neutralize or down-regulate the observed mutation.

In addition, even if the mutation of interest (i.e., a FGFR3 mutation) is not observed in a tissue sample, different mutations may exist in other genes that increase or decrease protein expression from the FGFR3 gene. Accordingly, detection of the FGFR3 gene product is a much more reliable indicator of cancer than detection of a mutation in the FGFR3 gene. A mutant FGFR3 gene may or may not have a direct impact on FGFR3 protein expression.

Consequently, there is a need for an improved method for the detection of bladder transitional cell carcinoma in a sample from an individual. A method based on the overexpression of FGFR3 protein as a diagnostic marker of bladder

transitional cell carcinoma, as disclosed in the current application, solves this problem. See, for example, Table 6 of the Application, reproduced below.

TABLE 6

	Total nº of samples	Useable sections	Positive cases	Negative cases	Null cas	% of positive cases**
Bladder transitional cell carcinoma pTa	37	36	25	11	1	**71.4 %**
Bladder transitional cell carcinoma pT1	100	93	67	26	7	**72 %**
Bladder transitional cell carcinoma pT2	72	67	33	34	5	**49.2%**
Bladder Healthy tissue	20	20	1	19	-	**5%**

As seen above, only 5% of samples of healthy bladder tissue are positive for FGFR3. In tissue samples from pTa and pT1 bladder transitional cell carcinoma, 71-72% of samples are positive for FGFR3. In tissue samples from pT2 bladder transitional cell carcinoma, 49.2% of samples are positive for FGFR3. Thus, FGFR3 protein expression is an effective indicator for bladder transitional cell carcinoma, particularly in pTa and pT1 carcinomas.

It is submitted that starting from the teachings of Capellen, the skillcd person would not have considered using the overexpression of FGFR3 protein as a diagnostic marker of bladder transitional cell carcinoma in order to solve the problem of detecting bladder transitional cell carcinoma. Upon measuring overexpression of FGFR3 protein what is directly observed is a change in the protein caused by mutations either in the own *FGFR3* gene or in other genes, or any other factor, that cause the overexpression of FGFR3 protein. Therefore, the

sensitivity and specificity, and, conscquently, reliability, of a method based on the overexpression of FGFR3 protein for detecting bladder transitional cell carcinoma is much higher than the sensitivity, specificity and reliability of a method based on the detection and identification of mutations in FGFR3 (Capellen).

In fact, Capellen teaches away from the current method, reporting that "[i]n all the samples with mutated *FGFR3, FGFR3b* mRNA levels were similar to or higher than those encountered in normal bladder and cervix epithelium (data not shown)." *Capellen, page 19, Col. 2.* If mRNA levels are **similar** in both states (normal and pathological) then FGFR3 mRNA levels cannot be used as a reliable marker of the disease. Capellen describes expression of an activated form of FGFR3 in tumors (*Capellen, page 19, cols. 1-2*), but Capellen does not clearly teach or describe that FGFR3, whether activated or not, is expressed at a much higher frequency in tumors, as demonstrated in Table 1 above. In fact, Capellen explicitly states that FGFR3 is detected in normal bladder cells ("Having detected *FGFR3* expression in normal bladder and cervix epithelia (data not shown), we examined the expression and mutational status of *FGFR3* in a series of bladder and cervix carcinomas to determine whether FGFR3 is involved in epithelial tumorigenesis." *Capellen, page 18, Cols. 2-3*).

The current application demonstrates that the percentage of bladder transitional cell carcinoma cell sampless expressing FGFR3 protein (49-72%) is

emphatically not similar to that seen in normal cells (5%), as seen in Table 6 above. This result would not have been anticipated, based on the teachings of Capellen.

Moreover, there is no relationship between the level of expression of the mRNA and the level of expression of its corresponding protein. This fact, which is general knowledge for the skilled person in molecular biology, is clearly demonstrated in Gygi et al., *Molecular and Cellular Biology,* 9 (3): 1720-1739 Mar 1999 (herewith enclosed), an article which had been cited 335 times in the scientific literature at the end of 2004.

Sturla cannot be relied on to correct the deficiencies in Capellen, as it is no longer available as prior art for reasons discussed above.

REJECTIONS UNDER 35 U.S.C. § 112

Claims 30-38 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Examiner argued that the specification does not enable determining the stage or severity of a bladder cell cancer. However, it is respectfully submitted that determination of the stage of severity of a bladder cell carcinoma is in fact enabled by the current application.

The specification clearly defines the severity of various stages of cancer, as follows:

> Bladder tumours are graded cytomorphologically from G1 to G3 in decreasing state of differentiation and increasing aggressiveness of the disease according to the World Health Organization (WHO). With respect to stage or invasivity, TCCs [transition cell carcinomas] of the bladder are classified as superficial papillary (Ta and T1),

muscle invasive (T2 to T4), or the uncommon carcinoma in situ or tumour in situ (TIS).

The method described in the specification is tested on four types of bladder tissue samples, including superficial papillary tumors (pTa and pT1; see Table 6 above); muscle invasive tumors (pT2); and non-cancerous cells. As seen in Table 6, 5% of non-cancerous cells expressed FGFR3; 71-72% of mild (i.e., superficial papillary) tumors expressed FGFR3; and about 49% of muscle invasive (i.e., severe) tumors expressed FGFR3. Based on the data in Table 6 above, a person of ordinary skill in the art would conclude that FGFR3 is associated with bladder cancer, and has a very small likelihood of being expressed in non-cancerous tissue (5%). Thus, if FGFR3 is detected, it is a very strong indicator that bladder cancer is present. FGFR3 is more strongly associated with mild forms of cancer (expressed in greater than 70% of tissue samples) than with severe muscle invasive cancers (expressed in less than 50% of tissue samples). Thus, a person of ordinary skill in the art would expect an increase in FGFR3 levels to be associated with formation of a superficial bladder tumor. By the same token, a person of ordinary skill in the art would expect a decline in levels of FGFR3 in a superficial bladder tumor to be an indicator that the tumor was changing into a more severe, muscle invasive tumor.

Based on the data in Table 6, a test for FGFR3 is positive for bladder cancer in 125 out of 196 samples of cancer tissue, and positive for bladder cancer in 1 out of

20 samples of normal tissue. The test detects bladder cancer with good sensitivity and high specificity, as shown below:

Immunohystochemical

	Negative	Positive
pTa+pT1+pT2	11+26+34=71	26+27+33=125
Control	19	1

Sensitivity (%)	63.78
Specificity (%)	95.00
Accuracy (%)	66.67
PPV (%)	99.21
NPV (%)	21.11
p-value	< 0.05

Similarly, a test for FGFR3 is positive for bladder cancer in 125 out of 196 samples of cancer tissue with milder tumors (pTa, pT1), and positive for bladder cancer in 1 out of 20 samples of muscle invasive tumors (pT2). The test distinguishes mild bladder cancer from severe bladder cancer with good sensitivity, as shown below:

Immunohystochemical

	Negative	Positive
pTa+pT1	11+26=37	25+67=92
pT2	34	33

Sensitivity (%)	71.32
Specificity (%)	50.75
Accuracy (%)	64.29
PPV (%)	73.60
NPV (%)	47.89
p-value	< 0.05

Accordingly, the current application enables use of the disclosed test to determine the stage or severity of a bladder cell cancer.

CONCLUSION

While we believe that the instant amendment places the application in condition for allowance, should the Examiner have any further comments or suggestions, it is respectfully requested that the Examiner telephone the undersigned attorney in order to expeditiously resolve any outstanding issues.

In the event that the fees submitted prove to be insufficient in connection with the filing of this paper, please charge our Deposit Account Number 50-0578 and please credit any excess fees to such Deposit Account.

Respectfully submitted,
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Date: July 15, 2010

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Exhibit A

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   British Journal of Cancer 92, 705-710 (1 February 2005) doi:10.1038/sj.bjc.6602384 Original Article
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2. **Author Index for Volume 89**
   British Journal of Cancer 89, 2355-2364 (12 December 2003) doi:10.1038/sj.bjc.6601554 Index
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3. **FGFR3IIIS: a novel soluble FGFR3 spliced variant that modulates growth is frequently expressed in tumour cells**
   L-M Sturla, A E Merrick, S A Burchill
   British Journal of Cancer 89, 1276-1284 (30 September 2003) doi:10.1038/sj.bjc.6601249 Molecular and Cellular Path
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